TREATMENT OF CHRONIC DIABETIC AND VENOUS ULCERS USING SAFE, LOW FREQUENCY (20kHz) AND LOW PRESSURE AMPLITUDE (55kPa) ULTRASOUND

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Abstract

TREATMENT OF CHRONIC DIABETIC AND VENOUS ULCERS USING SAFE, LOW FREQUENCY (20kHz) AND LOW PRESSURE AMPLITUDE (55kPa) ULTRASOUND

Sumati G. Nadkarni

The primary focus of this study was to assess the clinical efficacy of ultrasound assisted treatment of chronic wounds, such as venous and diabetic ulcers. The treatment was performed with fully wearable applicator, operating at 20 kHz and generating pressure amplitudes near 55 kPa. Over 500,000 patients are treated for venous ulcers annually and with treatment cost of $2,400 per month constitute approximately 1% of total health care costs in the western world. Due to the pain associated with these wounds, many patients are unable to meet their employment requirements after experiencing limited mobility and overall decrease in productivity. Hence, even modest shortening of the time needed for healing may provide substantial healthcare cost savings, and improvement of quality of life. The pilot study included 32 individuals between ages of 18 and 80 having venous (n=23) or diabetic (n= 9) wounds (~ 1cm^2 in size) that remained open for a minimum of 8 weeks. In compliance with the IRB study protocol the subjects were randomly assigned to either treatment or control group, with an equal chance of being assigned to receive active ultrasound treatment or sham (current standard care). Treatment sessions lasted 15 minutes and were administered once a week for a period of 12 treatments, or until the wound's closure. Clinical efficacy was evaluated by measuring the reduction in wound area over time. For both etiologies, i.e.

both venous and diabetic wounds the rate of closure was statistically faster ($p<.05$) in the treated group compared to the control group. The study findings show that the ultrasound treated venous ulcer group had statistically improved ($p<0.04$) rate of wound size change (reduction of 14.3%/week) compared to the rate of wound size change for the control group (increase of 3.6%/week on average). Diabetic wound closure was achieved typically after 4 sessions for treated wounds, as opposed to 7 sessions for the control group. Time to heal was also statistically faster ($p< .05$) for treated wounds (~5 weeks) when compared to non-treated wounds (~12 weeks). Overall, the results from this study support the notion that low frequency ultrasound treatment can successfully improve healing outcomes in chronic wounds with different morphology and etiology. The evaluated device used safe levels (<100mW/cm2 ISPTP) of ultrasound energy and featured unique portability, which opens possibility for personalized home treatment of chronic wounds in the future. The secondary goal of this study was to scrutinize statistical information available and develop guidelines to identify the most appropriate parameters suitable for initial (and limited) clinical trials characterized by relatively small sample size (n=32, such as the one discussed here). The outcome of the analysis indicates that minimum number of patients depends on the value of the desirable statistical power. Although this power can be selected by the user, the Food and Drug Administration sets a minimum value at 80% for clinical trials. It was found that most published literature lacks power analysis data and it is shown here that the number of patients is governed by a-priori determined p value, the difference in the assumed healing rate and biological wound variability. All these parameters must be carefully considered in the design of the clinical study.

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Chapter 1: Introduction

1.1 Problem Statement

This work centers on the efficacy of a novel treatment device, which uses ultrasound operating at 20 kHz and generating peak-to-peak pressure amplitudes close to 55 kPa to treat chronic wounds of the venous and diabetic type. A human study (n=32) was conducted at the Drexel University Wound Healing Center where patients were recruited in accordance with the Drexel University College of Medicine IRB. The following discussion offers insight into the clinical importance of enabling an alternative healing process to the standard chronic wound care currently offered, along with the succinct presentation of the methods, data and results from the clinical trial. In addition, it is the secondary aim of this study to discuss the proper parameters required to adequately interpret the results of a clinical trial.

Chronic wounds are a debilitating medical diagnosis that affect 6.5 million Americans every day, or roughly 1-2% of the U.S. population [5]. Due to the fact that chronic wounds can develop from multiple sources, no single treatment has been found to be effective. In 2009, chronic wounds carried with them an annual financial burden of roughly $25 billion dollars [5] and has been forecasted to increase. Deceptively innocuous from onset, these wounds can result from a small injury or infection, and persist for years without closure. A chronic wound is medically defined as any wound that does not responded to medical treatment and persists past 3 months [15].

Of the several types of chronic wounds, venous and diabetic ulcers are two of the most common pathophysiological types [1]. The sources of insufficient wound closure can include altered immunological status from a history of diabetes, infection, deficient

nutrition, obesity, immunosuppressive medication, and veins that lack sufficient ability to produce continuous blood flow and therefore leak to the surrounding areas, typically the legs. This induces edema as well as pooling of bacteria, which can cause open painful sores known as ulcers. [1]. Once an open wound has occurred bacterial infection, whether or not the cause of the ulcer, can opportunistically colonize the wound and substantially affect the prognosis. Staphylococcus aureus has been identified in more than 20-50% of chronic wound cases, and can become methicillin-resistant. Lastly, failure to close can actually be the result of a patient's natural inflammatory response. Rather than aiding in the healing process, excess edema caused by vasodilation can be counterproductive [1] by consistently stretching the wound, increasing surface area, and not allowing sufficient collagen and fibrous scaffolding – indispensable for healing process - to form.

The most common method of treatment for these wounds is debridement, which is discussed in more detail in Chapter 2. Current methods of treatment, also discussed in Chapter 2, are primarily passive treatments, and aim for the necrotic and infected tissues to be removed so that the natural immune response can fight the remaining infection. No active treatment exists, and – as evidenced below - the proposed ultrasound assisted device would be the first active form of treatment to advance wound closure.

Multiple factors contribute to chronic wounds, complicating treatment, and leading to an inability to standardize care. The most common current medical treatments involve (but are not limited to, as each wound is highly specific) antibiotic treatment, topical silver gels or foams (silver is a natural antimicrobial), pressure therapy, restricted movement of limb, encouraged elevation of limb, and as already noted, debridement. In recent medical history, continuous medical debridement has become the most popular

method to treat these wounds, as it has been empirically shown to result in shorter rates of closure [2]. Debridement, however, can be very painful as only a topical anesthetic is used and many patients suffer from deep and penetrating wounds, for which pain is not alleviated by the topical anesthetic. Some patients state that even controlled pain killers taken prior to treatment in anticipation of the pain do not alleviate the pain and therefore patient compliance is unsatisfactory due to avoidance of the procedure. Often, patients will skip appointments or not return until the ulcer has increased in size and is usually infected. [2]. Most debridement occurs in the doctor's office, however if the infection is not responsive or has penetrated the bone, surgical debridement, as mentioned, requires the patient to undergo surgery to remove deep layers of necrosis, gangrene, and bacteria. This is due to the fact that the patient must be under anesthesia to withstand the procedure, as the surgeon might need to debride all the way to, and including, bone tissue. Additionally, a skin graft might be used during this procedure to accelerate healing of the ulcer, should it occupy a large surface area. Often, at this point, osteomyelitis is a concern, and can only be scrapped out of the bone via surgery. Osteomyelitis is a bone infection, which is very dangerous because it is both hard to treat, due to its location within the bone, and can easily become systemic. Once systemic, the infection, if left untreated, becomes lethal. Currently, the only methods of treating osteomyelitis is to surgically remove the dead and infected bone and administer intravenous antibiotics for a minimum of 4-6 weeks [16].

Should no treatment prove effective, osteomyelitis occur, or the patient is unable to properly care for his/her wound, the last resort for the wound treated according to standard care approach is amputation of the limb. In addition to increased risk of

infection, complications of an extensive surgical procedure, and an already diminished quality of life, amputation of a limb has been well established by the psychiatric community to be a cause of severe anxiety, depression and even suicide (11). As such, it is imperative that wound management incorporates patient education on how to care for the wound, as well as identifies and develops a therapeutic method for accelerating wound healing that is painless, affordable, and easy to use. It is the first aim of this study, therefore, to show empirically that the device used here offers an alternative, yet effective solution to wound management practice. The proposed device should have the potential to activate healing mechanisms in persistent chronic wounds, but at the very least would increase wound healing rate in less obstinate wounds. The benefits of the former are clear, however the latter option would still be a significant improvement in the quality of life for patients suffering from chronic wounds. The pain and care associated with these wounds is debilitating and often leads to an inability to work, perform daily tasks, and restricts movement. Reducing the healing time returns patients to their former lives much faster, and also reduces the associated weekly cost burden and time spent at the doctor's office. The majority of patients must be followed by a physician between once a week to once every two or three weeks. For patients who are physically restricted by their wounds, this can be a huge burden. This device would be the first "in home" treatment, which would not require a doctor or nurse to administer the therapy. In addition to reducing the lifespan of the ulcer, it would introduce the first in home care system for autonomous use.

This clinical pilot study described in the following was designed to be randomized and to statistically determine whether or not the ultrasound device described has a

positive effect in wound closure, as compared to current wound management. Hypothesis testing is a reliable means of comparing and identifying correlated data but it can also be misleading or influenced to show data in a favorable light, when only a lesser correlation or no correlation exists. Pairing statistical significance with other supporting statistical factors provides more conclusive evidence that correlations found via hypothesis testing are in fact correct, and can thus provide a more complete picture of the associations in the data. The data was therefore analyzed for its statistical power in order to determine whether the remaining sample size is an appropriate representation of the population to support the findings. The statistics used to analyze the data will be further explored and explained in chapter 2.

While these additional parameters are commonly discussed in psychology and behavioral studies, they are rarely included in medical trials [3]. Most hypothesis testing compares two or more populations and uses a standard statistical test to determine whether or not there is statistically significant difference between the populations, as discussed in chapter 2. The hypothesis test chosen generates a p-value, which – in turn - determines whether or not this null hypothesis can be rejected, or fail to be rejected. Generally, most clinical trial studies only look at whether the p-value is less than or greater than the pre-chosen threshold, or alpha (generally .01, .05, or .10). A p-value less than the alpha value indicates that one should reject the null hypothesis, while a p-value equal to or greater than the alpha value indicates that failure to reject the null hypothesis. While usually sufficient to support clinical data, the focus on p-value outcomes limits the scope of information that may be revealed by additional parameters. This type of testing is most reliable when the sample size is large, and the data is normally distributed.

It is important to note that limitations of this study include sample size and variability. The patient population was derived from one clinic, and inclusion criteria excluded many candidates. Therefore the total sample size for this study was smaller than desired (see chapter 4). Sample size further diminished during the study due to drop out rates consistent with this population. One patient dropped out due to medical complications, and one dropped out due to inability to commute once a week to the doctor's office. It was also anticipated that there might be skew to the data due to the fact that each patient has a different size wound, different etiology, different level of self-care, and different medical background. While patients were instructed to follow doctors' orders, per usual, it is not possible to enforce, or verify whether this was happening. As such, it is reasonable to assume that the data might be skewed.

In order to correct for these anticipations, as well as to support findings of a small sample size, the power and effect size of the data are examined below. The power of the data will determine the statistical probability of a type II error, i.e. the probability of correctly rejecting the null hypothesis when the hypothesis is in fact false. This is important in establishing validity in the statistical data, as no data set realistically resides within a 100% confidence interval. Lastly, wounds treated by the sham device might also experience healing during the clinical trial. Linear trend lines were applied to each wound in each group, and the trend lines were analyzed. Trend lines provide a way of determining patterns in wound healing and can also normalize outlying data points, which may occur due to unforeseen events. A patient who, for example, was unable to tend to his/her wound one week might see changes in wound size that are incongruent with past healing. This might resolve in the following weeks, however the single week

can have a significant effect on the data. Trend lines normalize these outliers, and provide a smooth overall account of the behavior of the wound throughout the 12 applications.

This thesis is organized in the following way. First, the scope of this work, including the overall objectives and specific aims is outlined. Chapter 2 discusses the uses of statistics in research, as well as the proper interpretations of the statistics being used. Next, an overview of vascular disease and chronic wounds is provided in Ch. 3. Chapter 4 describes the human study treatment of diabetic and venous ulcers with ultrasound, presents the results and discusses the outcome. Lastly, Chapter 5 proposes future work and summarizes conclusions from the study.

1.2 Objectives

The overall goal of this research was to provide optimized statistical evidence that low frequency (20 kHz) low intensity ultrasound (55 kPa pressure amplitude corresponding to 100 mW/cm2) is an effective treatment for chronic venous ulcers, and that it can also be successfully applied to diabetic ulcers for wound healing.

Accordingly, the aims of the research were:

Specific Aim 1: Increase sample size and repeat study for venous ulcers using the parameters set by the previous research study.

Specific Aim 2: Introduce diabetic ulcers to the study to determine whether the ultrasound treatment can be extended to a different type of chronic wound with different etiology and immune response.

Chapter 2: Overview of Statistics in Research

This chapter discusses and summarizes the relevant statistical analysis used in this study, as well as facilitate understanding of the clinical data presented in chapter 3. It also reviews the importance of proper statistical analysis and delves into which statistics are relevant for illuminating results and why, as well as which statistics are often ignored by research.

2.1 The importance of proper statistical analysis

Statistical testing is an integral process in clinical trial studies. The purpose of a clinical trial is to either support or refute the medical claim of a certain drug or therapy and make an inference about the population at large based on a smaller sample of that population [10]. The best way to do this is to show the data from the trial, so it can be determined that the drug or therapy either improves the condition or does not improve the condition. Presenting raw data to the research consumer lacking context and defined study parameters, subsequently places the burden on the reader to draw conclusions from the study. This often results in interpretation errors and, therefore, a standardized method of analysis is needed to ensure that the purpose, intention, and results of a study can be properly weighed. Lack of, or erroneous, graphs' labeling also leads to misinterpretations. Data is relative, and not all correlations imply causation. A small change in e.g. glucose reading might be significant if the effects of a new insulin stabilizing drug are being assessed, whereas it might be insignificant in assessment of the effects of an insulin inducing drug. In short, context and parameters are essential to understanding just exactly what the data is trying to say, and whether or not there is clinically relevant effect.

Statistical understanding also contributes to the ability to analyze a study and determine whether or not proper protocol has been followed, and whether the results we are seeing make sense. The majority of published articles and journals make a claim one way or another. The claim can be taken as fact, or conclusions can be drawn based on the protocols defined in the study. If it is found that there might have been bias introduced in the study, or perhaps the population was not in fact randomly assigned, then it can be inferred that results might not be generalizable.

In addition to maintaining healthy dose of skepticism, being able to identify improperly conducted clinical trials can be a method of identifying unethical behavior in science [10]. For example, some studies might have had 10 patients, while the data only shows 5 or 15. The question becomes either what happened to the remaining 5 patients, or where did the additional patients come from. Often certain patients might not be included if they present "unfavorable results", however this has to be properly disclosed and explained, otherwise the analysis will be skewed and misrepresentative. The scientists' duty is to be proponents of prudent ethical conduct. In addition, if it is observed that a certain study has endangered or put undue risk upon its patients, it is a responsibility to flag such a fault.

2.2 Parametric vs non-parametric tests

Data can be defined in three major categories: numerical, categorical, or ranked. Numerical data are most common and it include data that have some numbers associated, such as heart beats per minute, or blood pressure. It is easy to quantify and characterize. Categorical data are data that have no natural order or ranking. Colors, without being assigned value, can be considered categorical. There is no numerical hierarchy, unless

assigned so, to colors. Ranked data are categorical data to which some ranking has been assigned. A second, and definitive, category for which data is characterized is the normal distribution, or whether the data is parametric and non-parametric. Data that is normally distributed is considered parametric data. Parametric data are those that can be described well by certain parameters [10], such as mean and standard deviation. Conversely, data that are not normal, skewed, or are hard to describe given parameters is called non-parametric data. In clinical trials, non-parametric data can arise in two scenarios; 1) the data are recorded as scores based on patient interpretation (pain scale) and 2) the sample size is too small, and there is not enough data to properly describe the phenomenon in the sample the way it appears in the population [10]. Once the type of data and the structure of the parameter being sought (number of variables, factors, and groups) is determined, the appropriate hypothesis test can be determined.

2.3 Student's *t*-Test

This study compared one variable (wound size) within two groups (experimental treatment vs. no treatment). When plotted and analyzed, the data was determined to be normalized and parametric. As such, the proper hypothesis test for this study was the Student's *t*-test. Had the data been non-parametric, a Wilcoxon rank sum test would have been more appropriate, as it better accounts for skewed data [18].

The Student's *t*-test is a hypothesis test which assumes normal data and compares two groups to see whether there is a difference in the means. In a t-test, the null hypothesis is typically set to assume that there is no difference in the two groups. The alternative hypothesis is then either one tailed or two tailed, as can be seen in figure 1 below [19].

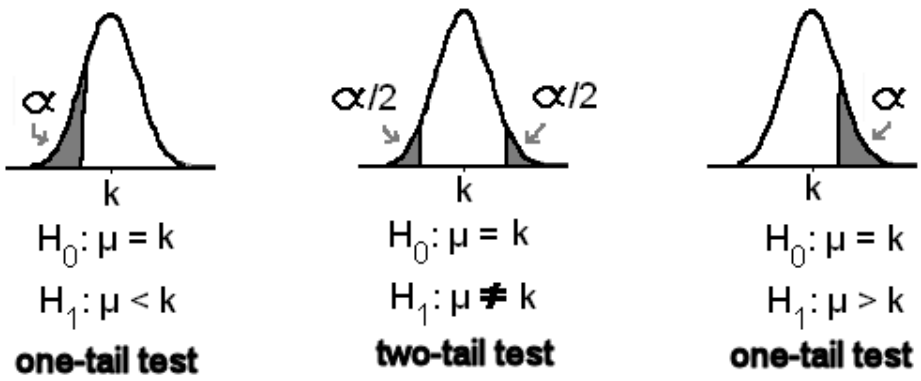

Figure 1. One and Two Tailed T-Test [19]

In a one tailed test, the mean of one group is assumed to be larger than the mean of another with good reason to support the 'one-way' assumption. For example, a group of 8th graders might be compared to a group of 11th graders on height. It would not be unreasonable to assume that the 8th graders will likely be shorter than the 11th graders, so a one tailed t-test would be appropriate. A two tailed t-test determines that there is a difference, but it is unclear where the difference might lie. For example, lowering the price a cookie might increase profit, because the cookie is less expensive and therefore more popular, or might decrease profit, because the cookie is not bringing in as much revenue. The effect on profit is unclear, and therefore in this situation a two tailed t-test would be appropriate.

A t-test can be paired or unpaired. A paired t-test means that one group has been put in two situations, or been given two interventions. For example, a group of 15 women were evaluated on the length of a headache without intervention. Several days later the same group was given an 81 mg dose of aspirin and evaluated again on the duration of the headache. The two data sets were then compared to see if the aspirin

reduced the duration of the headache. An unpaired t-test would use two different groups for each test, and would then compare the two groups for statistical significance. While a paired t-test might be ideal for some situations (avoiding bias or confounding affects), it is unrealistic in other scenarios.

The value that a t-test derives is known as the p-value. The p-value is the defining strength of an argument for or against the null hypothesis. In order to determine, however, whether or not your p-value is significant, one must determine at which percentage one wishes to assign cut off values, or significance. This is known as the alpha (α) and it is a user determined limit for which you compare your p-value, and is usually set to 0.05 or 0.01. Setting α to 0.05 means that there is a 5% chance that a significant p-value is incorrect, also known as Type 1 error. This would be akin to accepting a difference in the two groups, when one really does not exist. In clinical trials, an α of 0.05 is accepted as a reasonable cutoff, but can also be set to 0.01 for more precision, or any other value desired. The lower the alpha, the more precise a result is, however this also increases the chance of not finding a significance when one does in fact exist, or a type II error.

A type II error (β) occurs when a significant difference is not detected, when in fact one does exist. Two contributing factors to type II error include a sample size that is too small and the sensitivity of the difference you are looking to unveil. A larger sample size will reduce type II error, but it can also increase type I error. As such, a delicate balance is needed to minimize both types of error. While increasing sample size might decrease type II error, too many subjects can also be unethical, as it exposes more people unnecessarily to experimentation without increasing assurance [10]. It is the

responsibility of the researcher to determine the proper number of subjects necessary and to keep this number to a reasonable minimum. The above reasoning can be summarized in Table 1 below [13].

Table 1. Three Approaches of Hypothesis Testing [13]

Step	Test Statistic Approach	P-Value Approach	Confidence Interval Approach
1	State H_0 and H_a	State H_0 and H_a	State H_0 and H_a
2	Determine test size α and find the critical value	Determine test size α	Determine test size α or 1- α, and a hypothesized value
3	Compute a test statistic	Compute a test statistic and its p-value	Construct the (1- α)100% confidence interval
4	Reject H_0 if TS > CV	Reject H_0 if p-value < α	Reject H_0 if a hypothesized value does not exist in CI
5	Substantive interpretation	Substantive interpretation	Substantive interpretation

* TS (test statistic), CV (critical value), and CI (confidence interval)

2.4 Power

The power of a test, also known as the sensitivity, determines the probability that a difference is detected when there is in fact a significant difference. Power is defined as 1- β, and it increases as β decreases. Power can be an unappreciated parameter in research, and is often left unchecked. The consequence of not checking power leads to findings which appear determinant yet might instead be due to chance. Although power is a very informative and key parameter of statistical analysis Jacob Cohen, of NYU, reports that the majority of human studies ignore this parameter when reporting their findings [11]. This could be due to low power results, which conflict with reported findings, or due to lack of understanding of the parameter's significance. While a high powered study adds an element of confidence to the results, a low powered study can really only conclude that the sample population is not sufficiently indicative of the true

population mean. Table 2 below demonstrates power in comparison with other statistical values [13].

Table 2.Size and Statistical Power of a Test [13]

	Do not reject H_0	*Reject H_0*
H_0 is true	Correct Decision 1-α: Confidence level	Type I Error α: Size of a test (Significance level)
H_0 is false	Type II Error β	Correct Decision 1-β: Power of a test

It is a general consensus, as well as FDA regulation, that power should be greater than 80% in order to safely conclude that the hypothesis testing is accurate, and not due to chance [12]. If a study has a 50% power analysis, it is akin to flipping a coin and saying there is a 50/50 chance that the p-value is accurate. This relatively low – 50% - power can be increased by increasing the sample size, which reduces the chance that the occurrence is chance related. It is also important that the power is properly calculated. The parameters that go into the calculation can influence the outcome, and if using a statistical program, it is important to know which parameters to use. The parameters which should be properly assessed include the type of test being conducted (onemean, twomean, etc.), whether the test is one sided or two, the set alpha, and so on. Most programs will default to a two sided test, a power of 80%, and an alpha of 0.05. One sided tests have greater power than two sided tests, but can be used if it is reasonable to assume that the intended intervention has only one projected direction of outcome (increases or decreases).

The a-priori set parameters used in this study were:

- Alpha (α) set to = 0.05

- Power aim of 80%
- Unpaired Student's T-Test for average rate of closure (cm^2/week) for parametric data
- Two-Sample Comparison of Means for power analysis

In the next chapter an overview of the etiology of chronic wounds is given in order to understand the current diagnosis, differentiate the two wound types, as well as introduce the current passive treatment methods. This will hopefully illuminate the need for an active, portable and user-friendly treatment option, and the findings of this study can be better appreciated.

Chapter 3: Overview of Vascular Disease

In the following chapter the etiology and biology of venous and diabetic ulcers are discussed. Current standard of care for both diseases is also reviewed.

3.1 Venous Ulcers

Vascular disease is an all-encompassing term under which many etiological diseases fall. The vascular system is comprised of the body's network of arteries, veins, and capillaries. It stands as the body's network of blood vessels which carries blood and lymph throughout the body and is also known as the circulatory system. The purpose of the circulatory system is to deliver oxygen and nutrients throughout the body, while also removing waste and toxins. Damage to the vascular system can deprive organs or vital parts of the body of these nutrients, or can cause waste and pollutants to build up in a particular area.

There are many ways in which the vascular system can be compromised, including, but not exclusively, peripheral arterial disease, Raynaud's syndrome, varicose veins, blood clotting disorders, and venous insufficiency. Veins are vessels which bring blood back to the heart, and in the lower extremities venous blood flow is divided in to the superficial, communicating, and deep veins [6]. The communicating venous system connects the superficial venous system to the deep veins and all systems have one-way bicuspid valves that work with the leg muscles to allow blood to flow in a unidirectional manner back to the heart, avoiding reflux [6]. The pressure at rest in these veins has been determined to be about 80 mmHg in the standing position, and therefore the functionality of these valves is paramount [6]. Without proper functionality, the valves leak, and

backflow results. Backflow is the main trigger for the medical diagnoses discussed above.

Venous insufficiency, also known as venous hypertension, occurs when there is some pathology that is causing the proper flow of blood back to the heart to be compromised. There are four pathological mechanisms which have been identified in chronic venous insufficiency; 1) valve dysfunction due to existing or acquired conditions in the superficial or communicating veins, 2) valve dysfunction in the deep vein system due to congenital issue, weakness of valve, or damage to valve, 3) obstruction in the outflow of the deep veins, 4) failure of the pumping mechanism of the leg/calf [6]. It is important to note that there is no generally agreed upon sequence of events leading to venous insufficiency, or to venous ulceration.

Patients with venous insufficiency are at risk for many health hazards, including varicose veins, phlebitis, and venous ulceration. A venous ulcer is an open sore or wound which is a direct cause of venous insufficiency. Venous ulcers are not only painful, but can be dangerous, as they are a break in the protective skin layer, allowing for bacteria to have a direct path into the body. An elaborate scoring system has been put in place to help patients and physicians define the degree of severity of venous insufficiency and as well as to detail the urgency with which to seek treatment [17]. The CEAP scoring system (which stands for clinical, etiologic, anatomic, and pathophysiologic) has 6 designated levels, or categories, which are defined along with the recommended treatment, in the following way [17]:

Table 3. CEAP (clinical, etiologic, anatomic, and pathophysiologic) scoring system

Level	Diagnosis	Physician Recommendation
1.	Reticular and Spider veins	No need for medical treatment. Condition is purely cosmetic
2.	Varicose veins	Refer to a vein specialist for diagnostic testing routinely with no urgency
3.	Varicose veins and leg swelling	Refer to vein specialist quickly for diagnostic testing
4.	Varicose veins and evidence of venous stasis skin changes	Same recommendation as level 3
5.	Varicose veins and a healed venous stasis ulceration	Same recommendation as level 3
6.	Varicose veins and an open venous ulceration	Refer to a vein specialist urgently and to a wound care center for ulcer assessment

In addition to pain and complexity, venous ulcers are also a huge economic burden, costing the U.S. as much as $1.9 to $2.5 billion dollars a year [6]. The cost per patient includes home health care, hospitalizations, and dressings, which can add up to an average of $9,685 every three months [6]. This cost doesn't take into account the economics of salary forgone, disability, or forced early retirement. Unemployment and disability rates run high for those with venous ulcers, due to the fact that it is very hard to be on your feet and roughly 72% of persons who have a venous ulcer observe reoccurrence of ulcers in following years [6]. Additionally, 50% of patients have a venous ulcer for more than 1 year, and 34% have an ulcer more than 5 years [6]. It is

also estimated that 1% of people will develop a venous ulcer at some point in their lives, and this currently affects 500-600 thousand people today in the United States [17].

Treatment for venous ulcers include mechanical therapies, antibiotics, leg elevation, and debridement. Mechanical therapies include compression stockings, pressurized boots, and walking therapy. The goal of mechanical therapy is to aid the veins in properly pumping the blood and lymph away from the legs, allowing some healing to take place. While somewhat effective, mechanical therapies are considered passive, and do not heal the underlying causes. In a patient with a diminished immunity, or advanced venous insufficiency, this type of therapy can prevent further degradation, but rarely promotes healing. Antibiotics are used when there is an infection in the ulcer. Not all ulcers, however, are infected. As already noted, debridement, in combination with compression and elevation, is the most popular method of treating venous ulcers. Debridement involves physically removing the necrotic and gangrenous skin and bacteria on the top layers of the wound. This can be achieved in the doctor's office, or for more seriously infected wounds, via surgery in the operating room. Debridement is usually done with a scalpel and therefore can be very painful, even when topical analgesics are applied. While debridement currently observes the best results in wound care, patient compliance is relatively low due to the pain associated with the procedure. Should the ulcer not respond to treatment, or become dangerously infected, surgery to either graft an open wound, or amputate a limb, can be a debilitating last resort. The associated cost per day of a healing ulcer has been estimated to be 86$ a day, while the cost of a non-healing ulcer is much higher and harder to quantify [17]. In a recent study done in Massachusetts it was found that for healing ulcers, the average time to heal was 122 days, which is

roughly 17.5 weeks [17]. This amounts to a total average cost, at the very least, of $10,500.00 [17]. Reducing this time by 25% can save an individual $2,600, and reducing this time by 50% can save $5,200. Even a small reduction in healing time can have large long term impact on the economy.

3.2 Diabetic Ulcers

Diabetes is a metabolic disease in which a person's ability to regulate blood sugar becomes compromised due to either not producing enough insulin, or being unable to respond to insulin produced. An estimated 23 million Americans suffer from some form of this disease, and that is estimated to increase to 300 million worldwide by 2024 [14]. There are three types of diabetes; 1) diabetes 1, the autoimmune form where the body attacks the pancreas, 2) diabetes 2, the more common form in which the pancreas either doesn't produce enough, or the body becomes immune to insulin, and 3) Gestational diabetes, which affects pregnant women and can lead to insulin resistance. Risks associated with all forms include retinopathy (damage to the blood vessels in your eyes), neuropathy (damage to nerves), nephropathy (damage to the kidneys), heart disease and stroke. Neuropathy, damage to the nerves, can cause many unintended consequences, included injury and ulcers primarily found on the lower extremities. The economic burden of this portion alone of the diabetic healthcare epidemic is staggering. Diabetic foot ulcers and associated amputations alone were estimated to cost the U.S. healthcare payers $10.9 billion in 2001 [9]. It is estimated that 20% of all expenditures on the diabetes epidemic is directly for diabetic foot ulcers and amputations [9].

While appearing similar in presentation, diabetic ulcers come from very different etiologies than venous ulcers. Diabetic ulcers are largely neuropathic ulcers that form in

patient with diabetes mellitus [6]. While some diabetic patients do present with vascular insufficiency, 70% of diabetic ulcers are due to neuropathy, where pressure points develop from thickened calluses, and a breakdown occurs in the tissue forming an open sore. Diabetic ischemia can cause the skin to be less resistant, and to therefor breakdown more easily. Ischemia also reduces protective sweating in the feet, allowing for skin to become dry and thin [7]. As such, the most common cause for diabetic ulcers is due to physical trauma or accidental abrasion that causes tears or wounds of fragile tissue [7]. Neuropathy in the feet also means that such abrasions may not be felt or noticed at the onset, or could be caused by lacking ability to control or feel placement of the feet. Ulcers which precipitate long before the patient is aware of the wound cause even worse damage than those which are known from the onset. The outcome of this unidentified problem on a larger scale is that the lifetime risk of having a diabetic ulcer for any diabetic patient is roughly 15% [7], while the rate of reoccurrence of an ulcer is greater than 50% after three years. [9]

Like venous ulcers, diabetic ulcers can also be predisposed to bacterial infection. This causes additional complications in treating the wound and substantially delayed healing. Untreated, unknown infected wounds could progress into osteomyelitis (infection of the bone), become systemic or cause thrombosis, all of which can be lethal. Reduced phagocytosis and bactericidal capacity in diabetic patients are common immunodeficiency issues in diabetic patients. This means they are more susceptible to infection, and once infected the health risks are severely increased compared to someone with a normal immune system [8]. Due to the possible risks, diabetic ulcers are far more

likely to end up in amputation or surgical intervention. Roughly 15-27% of these ulcers require some degree of surgical removal of bone [7].

Treatment of diabetic ulcers, however, is very similar to the treatment of venous ulcers. In addition to antibiotics, mechanical pressure, elevation, and debridement, diabetic patients are encouraged to monitor their blood glucose levels and maintain proper diets. Proper control of their diabetes is paramount for supporting their immune system and for reducing neuropathy. Additional weight distributing shoes might be used to offload pressure points caused by regular street shoes [8]. Keeping the skin around the wound, and on the lower limbs, moisturized is also targeted, as it can keep the organ from becoming thin and brittle.

In the next chapter the study of ultrasound assisted wound healing will be presented. The study parameters will be outlined and the methods and materials used will be described. Also, the results of the clinical pilot study will be introduced, along with their analysis and interpretation.

Chapter 4: Venous and Diabetic Treatment with Ultrasound Applicator

This chapter outlines the study from description of the ultrasound applicator, to analysis of the data. It begins by describing the device and preset requirements. It then describes the protocol and procedure, and analyzes and describes the data obtained. Lastly, a discussion for each wound type summarizes the data analysis, delves into complications observed, and describes correlations found.

4.1 Introduction

As discussed in chapter 3, chronic venous and diabetic ulcers are a painful and costly burden that affect 6.5 million Americans each year [5]. While debridement, compression therapy and constant monitoring are the current means of management, no device or treatment exists to treat these wounds in a pro-active way. A previous pilot study, "low-intensity (<100 mW/cm^2) ultrasound to treat venous ulcers: A human study and *in vitro* experiments" [4] determined that the low frequency, low intensity, ultrasound treatment was in fact able to accelerate healing of chronic venous ulcers. This pilot study was revisited with the aims of recruiting a larger sample size, as well as expanding the patient base to include chronic diabetic ulcers. In this chapter the study will be described in detail, as well as the results, which not only reinforce the previous studies results, but successfully demonstrate that this device can be extended towards treatment of diabetic ulcers.

The ultrasound applicator used here is described below

4.2 Materials and Methods

Therapeutic Tool: Ultrasound Applicator

The device, or ultrasound applicator, used in this work in active treatment of the chronic wounds has been previously described in Sunny *et al. (2012)* and Samuels *et al (2013).* Briefly, the applicator employs flexural transducers powered by a custom designed rechargeable battery unit [4]. The device that generates acoustic energy can be designed with differently shaped geometry to tailor individual wounds. In the renditions below the left hand side (LHS) device (Fig. 3) is fabricated as 40 mm dia disk comprising

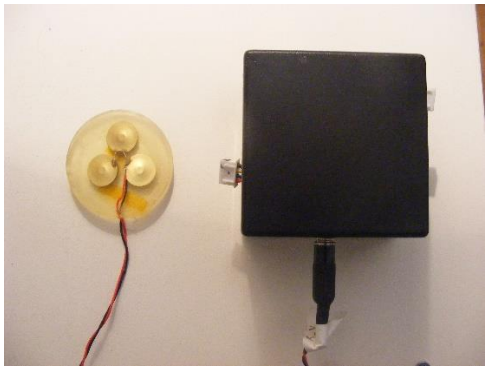

Figure 3. Circular, 40 mm diameter, 10 mm thick disk design with three single elements

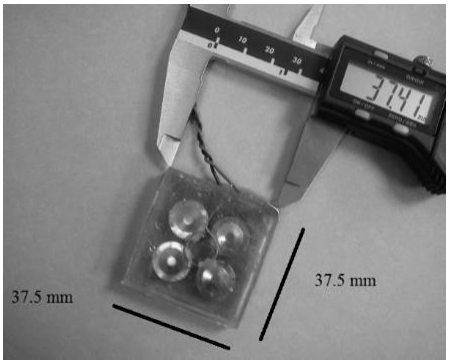

Figure 2. Rectangular (37X37X10 mm) design with 4 active elements

of three individual elements, whereas in Fig 4 (RHS), the low weight (<100 g) applicator was designed as four elements device having dimensions (37mm X 37mm X 10 mm).

The acoustic output of the active device was previously determined by Samuels *et al (2013).* The intensity of the device has been set to ~100 mW/cm^2, spatial peak temporal peak, and the frequency to ~20 kHz. These parameters were chosen due to their maximum efficacy and their ability to have the greatest impact on the rate of wound

healing (see also section discussion). They were derived from the study previously mentioned by Samuels et *al*. The acoustic output was checked and calibrated once a week to maintain consistent output and effectiveness.

Patient Selection

Patient selection followed and was approved by mandatory IRB regulations and review. All patients were recruited by obtaining informed consent, and were free to withdraw from the trail at any time. They were compensated $25 dollars each treatment, in voucher form to be redeemed for cash at the hospital cashier. Recruitment for the study was from the Drexel University Wound Healing Center, and included specific criteria in order to standardize the type of wound being studied. There were two ulcer types involved in the study, and all ulcers were classified as CEAP level 6. Ten diabetic and ten venous ulcers, totaling 20 patients were recruited. Each type included a treatment and a sham group (5 in each group). The study was single blind (the researcher administering the treatment knew whether the patient was being given the treatment or sham, while the patient was not). While an ideal study would have been a double blind study, which would further reduce any possible bias, due to the limited time allocated for patient visit and the fact that only one person was available to administer the treatment it was decided to permit the study to be performed as a single blind one. Patients were recruited, and then assigned a number. The numbers were then randomly assigned the treatment or sham device. Inclusion criteria for participation included the following:

- Patients between the ages of 18-80 years old

- An ulcer at least 2 months old
- An ulcer size of approximately 1 cm^2 or greater
- No known allergies to Tegaderm (plastic sterile wrapping for ultra sound). (see figure 4 below)

Patient Treatment

The duration and frequency of the treatment was also established by the previous patient study for maximum effectiveness [1]. The treatment procedure began with a 1 in. green marker placed alongside the wound followed by three pictures taken (in the macro setting) of the wound and marker for later digital analysis (after a nurse has removed dressings and applied lidocaine, if applicable).

Next, a sterile ultra sound gel was placed directly on the wound to enhance conductivity of the sound waves to the wound. The ultrasound applicator was wrapped with a sterile Tegaderm plastic coating, and then placed directly on the gel (Aquasonic 100) and over the wound. See Figure 4 below.

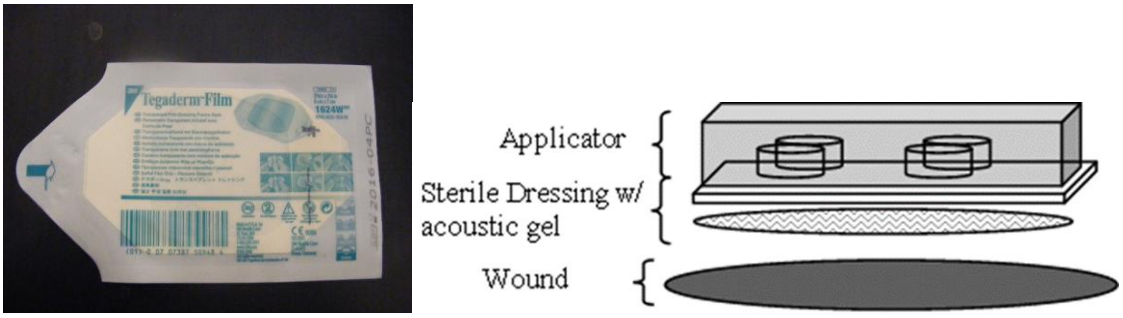

Figure 4. Tegaderm Film and Order of Application to Wound

To secure the device, medical tape was placed over the device and adhered to the skin. Care was taken not to place the medical tape over the wound, or additional wounds in the vicinity. The active device was turned on for 15 minutes, and then shut off and removed from the patient. The ultrasound coupling gel was gently wiped off using a sterile 4X4 gauze, and then lidocaine was applied again if desired. After treatment the doctor consulted the patient and debrided the wound as per usual. Treatments took place once a week for 10-12 weeks, or until closure of the wound. All patients, regardless of group, continued to receive normal standard of care after the treatment had been administered. This included assessment form the doctor, debridement and cleansing of the wound.

Wound size determination: digital camera

One of the three pictures obtained in each treatment was used to analyze the trends of the wounds per week. A properly captured picture displayed the full wound, head on, as well as the green 1 inch marker, also head on, as seen below in figures 5 and 6.

Figure 5. Improper Picture Capture – Off Center

Figure 6. Proper Picture Capture – On Center

The green marker can be susceptible to appearing less green, or washed out, if a flash was present, so a proper picture displays the green marker as not washed out or faded. Improper flash can be seen below in Figure 7.

Figure 7. Improper Picture Capture - Faded Marker

One picture from each treatment session was chosen and using a stylus and a touch screen computer (*Lenovo Yoga 13)*, the area wound was traced in Microsoft™ Paint using a specific color code (164, 255, 0). The picture was then saved as a bitmap file and stored in folder designated for traced pictures. A MatLab program, designed

specifically for the previous study, was used to calculate the wound sizes in each picture. A report was generated from the MatLab Program, which listed the wounds by file name and area (cm^2). From this data, the area of the wounds was compiled and analyzed. A trend line was applied to each patient's wound data as well, and from this the rate of healing was extrapolated.

4.3.1 Results and Statistical Analysis for Venous Ulcers

This study included 10 venous ulcer patients of CEAP (clinical, etiologic, anatomic, and pathophysiologic) class 6. No venous ulcer patient dropped out of the study, and therefore the data is inclusive of all 10 patients. Of the 10, 5 were treated with the device, and 5 were treated with a sham device (no treatment). In both sham and treatment group, 2 patients healed completely, while 3 patients did not heal completely by the 12^{th} treatment. One patient, however, healed after one treatment, and was therefore excluded from analysis due to preset exclusion criteria of requiring at least 2 treatments to be considered device related. Due to (relatively) low sample size, this data was combined with 16 patients from the previous trail. Hence in total there were 25 patients' data considered in the venous ulcer study, 12 receiving sham treatment and 13 receiving treatment

The wound sizes were normalized for relative comparison, and the average percent of wound size change per week was determined. Figure 8 below demonstrates that the treated venous ulcers decreased in size by roughly 14.3%, while the non-treated venous ulcers increased in size by 3.6% per week. This indicates that the ulcers which

were treated by the ultrasound were healing, while those that were not treated with the ultrasound were getting larger and deteriorating.

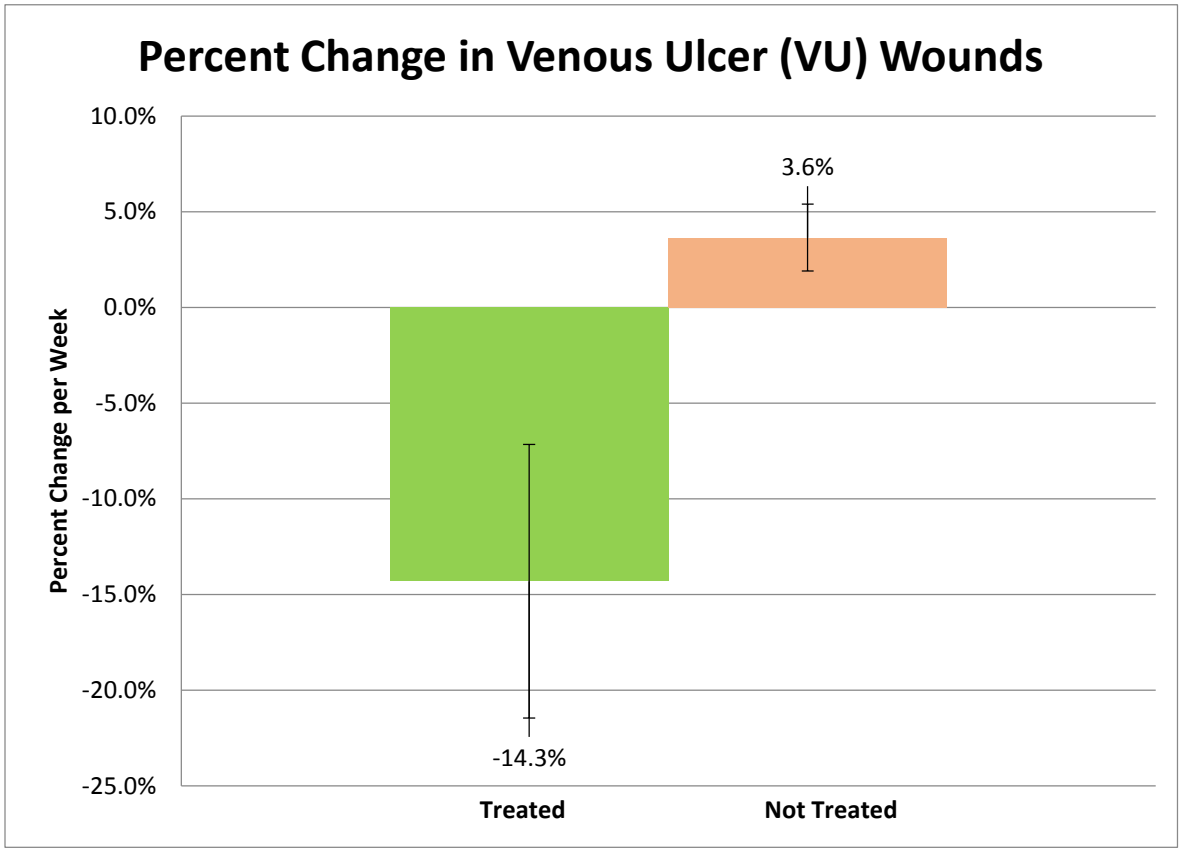

Figure 8. Percent Change in Venous Ulcer (VU) Wounds. This graph demonstrates that the venous wounds which were treated with the ultrasound closed on average 14.3% per week, while those which were not treated grew larger by 3.6% per week.

The initial wound sizes were found to be normally distributed, and were thus hypothesis testing was conducted using a Student's T-test (see chapter 2). In addition, the p-value of the wound size comparison was 0.566, which indicated a failure to reject the null hypothesis ($h_o \geq 0.05$) and designated that the two groups had no significant difference in size. The t-test comparing the treatment and non-treatment group healing rate provided a p-value of 0.0023 ($p < 0.05$). This indicated a statistical significance

between groups in rate of healing. This supports the findings in figure 8, which show that the treatment group had a mean rate of healing which was statistically different from the non-treatment group. See Table 4 below.

One-sample t test

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
woundh~e	13	-7.206154	2.972877	10.71886	-13.6835	-.7288107

mean = mean(woundhealingrate) t = -3.4802
Ho: mean = 3.14 degrees of freedom = 12

Ha: mean < 3.14
Pr(T < t) = 0.0023

Ha: mean != 3.14
Pr(|T| > |t|) = 0.0045

Ha: mean > 3.14
Pr(T > t) = 0.9977

Table 4. Venous Ulcer One-Sample t-test

The power analysis done in STATA13™ yielded a power of just under 60%. Unfortunately, ideal power for a clinical trial is 80%, and while this does not negate the findings, it does increase the probability of type II error (β) to 40%. STATA13™ was also used to calculate the ideal n for a power of 80%. The program determined that each group would require n=28. See output below.

```
Estimated power for two-sample comparison of means

Test Ho: m1 = m2, where m1 is the mean in population 1
                  and m2 is the mean in population 2
Assumptions:

         alpha =   0.0500  (one-sided)
            m1 =     3.14
            m2 =   -7.206
           sd1 =    16.64
           sd2 =    10.72
sample size n1 =       12
            n2 =       13
         n2/n1 =     1.08

Estimated power:

        power =   0.5740
```

Output 1. Power Analysis for Venous Ulcers

```
Estimated sample sizes for a two-sample means test
Satterthwaite's t test assuming unequal variances
Ho: m2 = m1  versus  Ha: m2 < m1

Study parameters:

        alpha =    0.0500
        power =    0.8000
        delta =  -10.3460
           m1 =    3.1400
           m2 =   -7.2060
          sd1 =   16.6400
          sd2 =   10.7200

Estimated sample sizes:

            N =        48
N per group =        24
```

Output 2. N desired for 80% Power

For additional statistical analysis the odds ratio was calculated comparing whether overall healing was observed in the treated group vs. the non-treated group. Observed healing was defined as a negative healing rate in cm^2/week. A positive healing indicated that the wound was increasing in size overtime and was therefore considered a non-healing wound. This odds ratio conducted in STATA13™ reported the following:

	Exposed	Unexposed	Total	Proportion Exposed	
Cases	9	5	14	0.6429	
Controls	2	7	9	0.2222	
Total	11	12	23	0.4783	
	Point estimate		[95% Conf.	Interval]	
Odds ratio	6.3		.7171192	78.33136	(exact)
Attr. frac. ex.	.8412698		-.3944682	.9872337	(exact)
Attr. frac. pop	.5408163				
	chi2(1) =	3.88	Pr>chi2 = 0.0487		

Output 3. Odds Ratio for Venous Ulcers

This odds ratio reported a p-value of 0.0487, which was significant ($p < .05$). This further supports the evidence that there are more healing venous ulcer patients in the treatment group, compared to the non-treatment group.

4.3.2 Results and Statistical Analysis for Diabetic Ulcers

There were 10 patients in the diabetic ulcer group at the beginning of the study. One patient dropped out of the study after a heart attack and medical complications, and another patient dropped out of the study after two applications for unknown reasons. Of

the remaining 8 patients, *all* patients demonstrated a healing trend. The t-test concluded that the average rate of closure was statistically significant ($p<0.5$) with a p-value of 0.0104. The weeks to closure was also found to be statistically significant ($p<0.05$) with a p-value of 0.0291, and the initial wound size was determined to be not significant ($p>0.05$) with a p-value of 0.6075.

One-sample t test

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
woudsi~e	4	-31.915	5.640616	11.28123	-49.86596	-13.96404

mean = mean(woudsizerate) t = -4.4738
Ho: mean = -6.68 degrees of freedom = 3

Ha: mean < -6.68	Ha: mean != -6.68	Ha: mean > -6.68
Pr(T < t) = 0.0104	Pr(|T| > |t|) = 0.0208	Pr(T > t) = 0.9896

Output 4. Diabetic Ulcer One sample t-test

Of the treated patients, the average weeks to closure was 4.67, while the average weeks to closure for non-treated patients was 12, as shown in Figure 9 below. This indicates that treated wounds closed 3 times faster than non-treated wounds, and saved an average of 8 weeks of treatment. Average treated sessions to closure was 4 for the treated group, and 7 for the non-treated group.

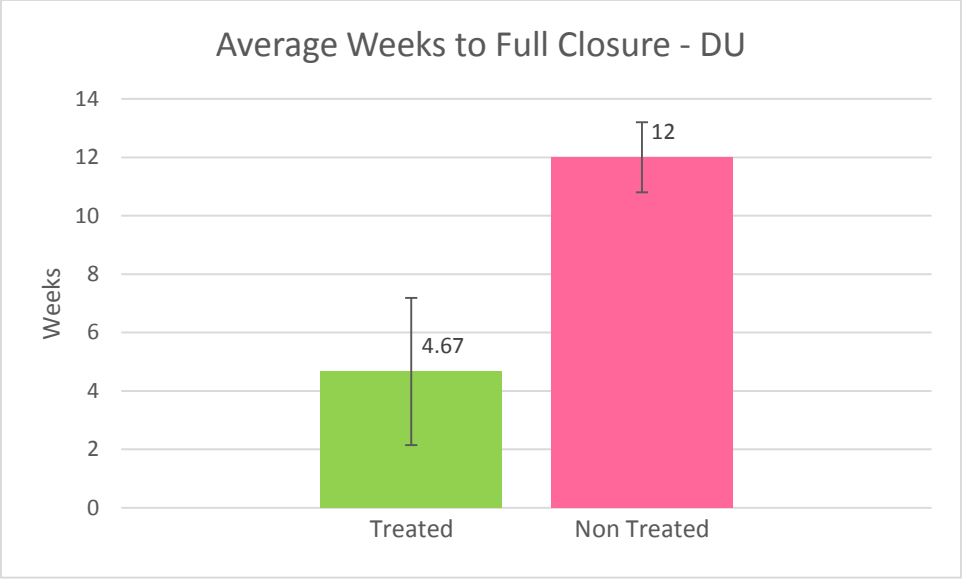

Figure 9. Average Weeks to Full Closure for Diabetic Ulcers (DU). This graph shows that treated diabetic ulcers healed 3 times faster than non-treated diabetic ulcers.

The wound sizes of the diabetic wounds were also normalized for comparison, and the percentage change in wound size per week was computed. If a patient missed a day, or more, the projected wound size was included in order to properly normalize the data. Figure 10 below shows the average percentage wound change per week for diabetic patients. The treated group's ulcers healed at an average rate of 36% per week, while the non-treatment group healed 16% per week.

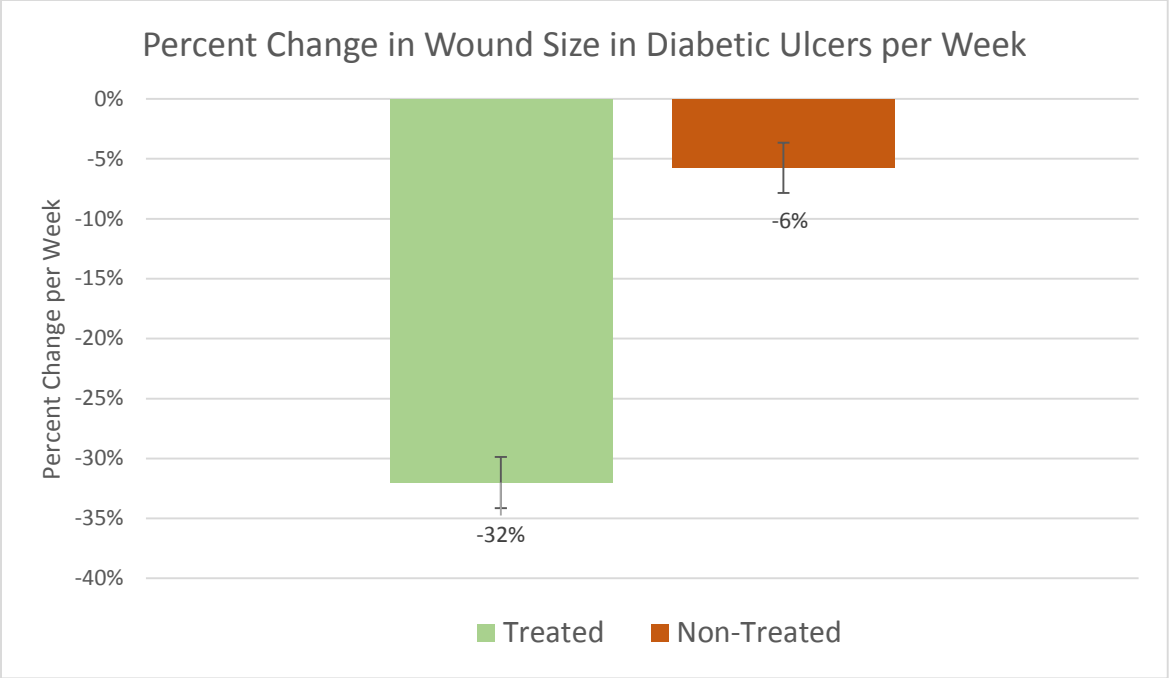

Figure 10. Percent Change in Wound Size in Diabetic Ulcers (DU) per Week. The treated diabetic ulcers closed 5 times more than the non-treated diabetic ulcers.

The power analysis for the diabetic wounds, while having less patients in each group, observed higher power than anticipated. The power analysis was run in STATA13™ and was determined to be roughly 99%. While unexpected, this is a positive finding as it states that there is a 1% chance that the true correlation found by the hypothesis testing is in fact erroneous. It can therefore be said with 99% confidence that the t-test was accurate in finding a strong correlation between treatment and healing. The number of patients needed to have 80% power analysis was also computed via STATA13™. It was found to be 3 patients per group. Power analysis, along with all relevant parameters, is given below.

```
Estimated power for two-sample comparison of means

Test Ho: m1 = m2, where m1 is the mean in population 1
                  and m2 is the mean in population 2
Assumptions:

         alpha =   0.0500  (one-sided)
            m1 =    -6.68
            m2 =  -31.915
           sd1 =     4.95
           sd2 =    11.28
sample size n1 =        4
            n2 =        4
         n2/n1 =     1.00

Estimated power:

        power =   0.9929
```

Output 5. Power Analysis for Diabetic Ulcer Patients

```
Estimated sample sizes for a two-sample means test
Satterthwaite's t test assuming unequal variances
Ho: m2 = m1  versus  Ha: m2 < m1

Study parameters:

        alpha =    0.0500
        power =    0.8000
        delta =  -25.2350
           m1 =   -6.6800
           m2 =  -31.9150
          sd1 =    4.9500
          sd2 =   11.2800

Estimated sample sizes:

            N =         6
 N per group =         3
```

Output 6. Diabetic Patient Desired N for 80% Power

4.3.3 Discussion

The results obtained for both populations' supports the initial hypothesis that low frequency (20 kHz) and low intensity (<100mW/cm2 ISPTP) ultrasound aid the healing mechanism in chronic diabetic and venous ulcers. Due to low ($n < 30$) patient population access in the clinic and 90% patient retention due to the nature of a clinical trial (comorbid health related occurrences and scheduling conflicts), the population for each group was lower than needed for significant power ($P > 80\%$). This proved to be an issue with the venous population, as the results recorded, while promising, were underpowered by about 20%. It did not, however effect the power of the diabetic patient population. This is due to the fact that the healing rate in the diabetic treatment population was 4 times faster than that of the non-treatment group, which further supports the data obtained. Initially, however, it was hypothesized that the diabetic group would exhibit reduced response to the ultrasound as compared with the venous group, due to the compromised immunities of the diabetic patients.

While the immunity of diabetic patients may have had negative healing affects compared to the patients with venous ulcers, this was not elucidated in the results. On the contrary, while all patients in the diabetic group, whether treated or not, healed by the end of the 12^{th} treatment, only 60% of the venous ulcer patients healed by the last treatment. Patient compliance and attendance was much greater in the diabetic ulcer group, compared with the venous ulcer group, which could be one reason this discrepancy was detected. It was also observed that patients who had diabetic ulcers appeared more motivated in the care of their wound, and had higher levels of compliance with the treatment. Possible reasons for this observance include 1) heightened awareness of their

health due to living with an autoimmune disease, 2) having had previous amputations and therefor seeing amputation as a life altering and terrifying prognosis, and 3) those with ulcers on their feet were not able to walk at all, and therefor were able to resist putting pressure on their ulcers and thus allowing them to heal. Other explanations for increased motivate, which might be more personal and unknown are also possible.

The initial wound sizes of each group, while not statistically significant within their own populations, were 7 times greater on average in the venous population. Diabetic wound sizes averaged about 1.0 cm^2 while venous ulcer wounds averaged 7.0 cm^2. This discrepancy did not affect the group analysis individually, but could be a reason why the diabetic wound were all able to heal within the 12 treatments, while the venous ulcers were not. Had the venous ulcers been smaller at the onset, they might well have healed within the 12 weeks. Alternatively, a longer investigation of at least 24 weeks (2X the current time) would be necessary to make further conclusions.

As discussed above, the power analysis for the venous ulcer group was 60% (lower than expected). With an n=25, when combined with previous data, it was anticipated that the power would be greater than 80%. There are several possible reasons for this. First, it is possible that the positive correlation between healing and treatment was incorrectly identified from the hypothesis testing. This possibility, however, is not likely due to the multiple tests performed and the consistency of results. Another possibility is that the venous ulcer group data had several anomalies, compared with the diabetic data which was more consistent. Patient anomalies include the following:

Patients 5 and 20, both non treatment subjects, took 17 weeks to complete the 12 treatment sessions, with several gaps in between sessions. While they both were documented as healing by the 8th session, had they been compliant in their care, they both would have had different healing rates as compared with patients who were consistent. Protocol, however, dictates that we document 12 sessions, not 12 weeks, so their data was included.

Another inconsistency involved patient number 10, a treatment patient whose wounds visibly reduced, however picture analysis showed an increase in wound size. Patient 10 was confined to a wheel chair and the wound was located under the right leg. Due to the size of the wound at onset, and the inability to maneuver the leg, pictures were often taken at an angle, or cut off. This corrupted the analysis of the patient's data, and possibly skewed the results.

Patient number 8 was another patient who had complications during treatment. This patient, who suffered from a genetic blood clotting disorder, stopped taking prescribed blood thinners half way through treatment and suffered a pulmonary embolism a week later. This lead patient 8 to be hospitalized and unable to care for the wound for a full week. It also likely impacted patient 8's immune system, reducing the rate of healing. Patient 8's wound had initially showed signs of healing up until this occurrence, but then proceeded to remain unchanged. It is likely that this patient's data was biased and not fully representative of compliant care.

The venous ulcer group showed greater variability in its healing trends, and therefore further analysis was conducted to find additional trend associations. The rate of

healing per weak was calculated and normalized to obtain a relative comparison. The groups were divided into healing patients and non-healing patients, and then compared between treatment and non-treatment groups. Figures 11 and 12 below show the average healing rate percentages per week for both healing and non-healing patients.

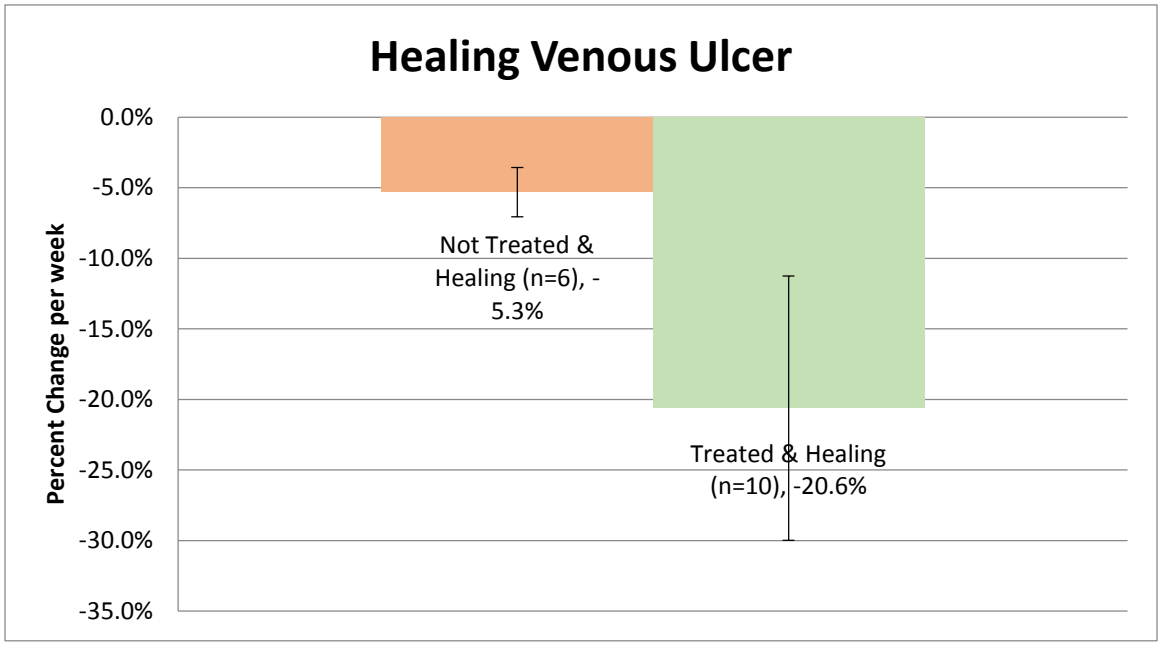

Figure 11. Percent Rate of Wound Healing in Venous Ulcer (VU) Patients per Week. The wound size of the healing treated venous ulcers decreased 4 times more each week than the non-treated ulcers.

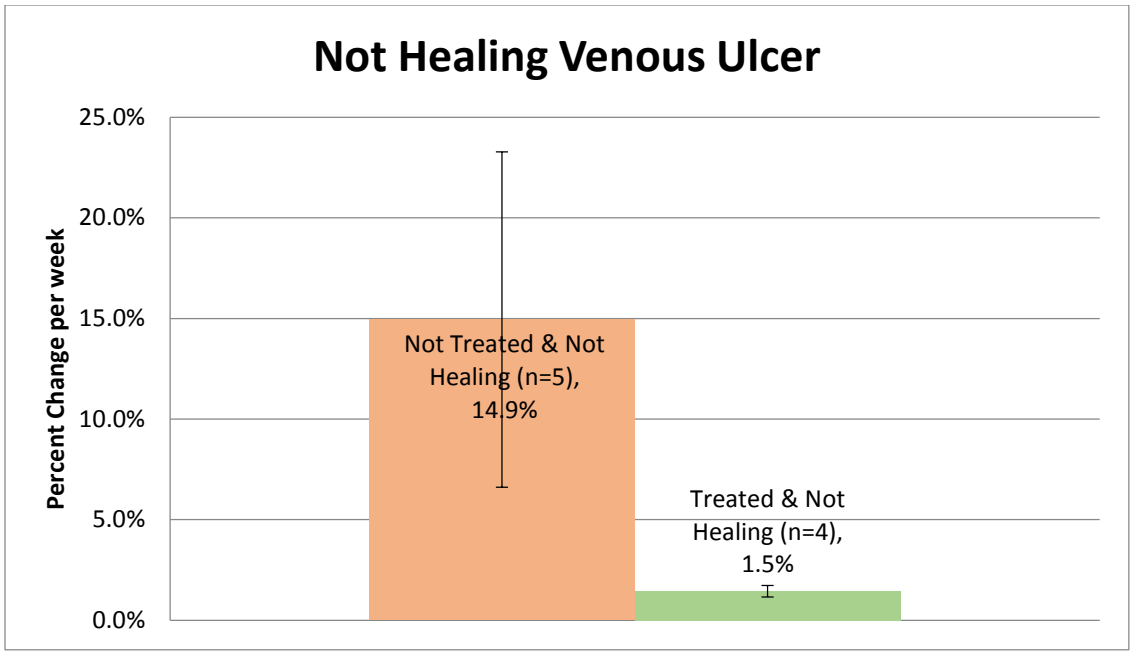

Figure 12. Percent Rate of Wound Healing in Non-Healing Venous Ulcer (VU) Patients per Week. In the non-healing ulcers, the treated VU's grew in size by 1% compared to the non-treated venous ulcers. This shows that even in resistant ulcers, the ultrasound provides some protective measure to reduce the rate of deterioration.

As can be seen in Figure 11, the treated patients had a percent wound size change at almost 4 time greater than the non-treated patients, when observing only the patients whose wounds were showing signs of healing. For the patients whose wounds were not healing, the percent change in wound size per week was almost 10 times less for the non-treated patients than the treated patients. This analysis is significant because it shows that even in patients who appear to not be responding to treatment the wounds are not increasing as much as the wounds that are untreated. It is possible that the device is in fact working on these wounds, which appear to not be responding, and are resisting growth rather than healing, which would be the alternative as indicated by the non-treatment group.

Additional power analysis was conducted to see if these patients (originally included in the analysis) impacted the results of the venous treatment group. The results showed an increase in power by 4%, which was an improvement, but not as much as anticipated. This, of course, is due to the fact that while the results were better, the group had two fewer subjects, which impacts the power of the test.

Using the above parameters, and excluding the outlier patients, the number of subjects required to achieve 80% power was once again computed. With the increased results from more strict exclusion criteria, the number of subjects reduced from n=24 to n=19. This proves that excluding the wounds, which were anomalies, and patients who had significant medical complications, the study could reduce the overall number of patients recruited.

```
. power twomeans 3.14 -8.56, sd1(16.64) sd2(11.14) n1(12
> ) n2(11) onesided

Estimated power for a two-sample means test
Satterthwaite's t test assuming unequal variances
Ho: m2 = m1  versus  Ha: m2 < m1

Study parameters:

        alpha =    0.0500
            N =        23
           N1 =        12
           N2 =        11
        N2/N1 =    0.9167
        delta =  -11.7000
           m1 =    3.1400
           m2 =   -8.5600
          sd1 =   16.6400
          sd2 =   11.1400

Estimated power:

        power =    0.6105
```

Output 7. Power Analysis without Outliers

```
. . power twomeans 3.14 -8.56, sd1(16.64) sd2(11.14) onesided

Performing iteration ...

Estimated sample sizes for a two-sample means test
Satterthwaite's t test assuming unequal variances
Ho: m2 = m1  versus  Ha: m2 < m1

Study parameters:

        alpha =    0.0500
        power =    0.8000
        delta =  -11.7000
           m1 =    3.1400
           m2 =   -8.5600
          sd1 =   16.6400
          sd2 =   11.1400

Estimated sample sizes:

            N =        38
  N per group =        19
```

Output 8. Desired N for 80% Power without Outliers

Lastly, in order to have fully supporting data, another investigative clinical trial with more patients would need to be performed. As already indicated, the FDA approval (510k) process requires minimum 80% power analysis using $p < 0.05$ to accept/approve the hypothesis used here (i.e. that Drexel applicator applied as described accelerates and fully heals chronic wounds) [12]. While the diabetic ulcer population was well within these limits ($\beta=0.01$), the venous ulcer population was shy of these power requirements. To achieve such power STATA™ 13 assisted analysis indicated that a minimum of 38 venous ulcer patients will have to be treated (19 treated and 19 sham). This number needs to be augmented by 25% based on our knowledge of patients' retention rate. Hence the total number of the patients needed to complete this study is 48 (24 treated and 24 sham)

venous ulcer- and 20 diabetes ulcer patients to bring the DU study up to roughly 30 patients, or the generally agreed upon value for a statistically 'large sample'. Corresponding numbers to satisfy a 90% power analysis will require a minimum of 52 venous ulcer patients (26 treated and 26 sham patients). Again, this number should also be augmented by 25% to account for patient retention rates. Hence the total number of patients needed to complete this study with 90% power would be 66 venous ulcer patients (33 treatment and 33 sham) and 20 diabetic ulcer patients. A summary of these values can be seen in table 4 below.

Ulcer Type	N In Study	Power of Study	Desired N for 80% Power (before dropout rates)	Desired N for Future Study
Venous Ulcer	23 patients	58%	48 patients	66 patients
Diabetic Ulcer	9 patients	99%	3 patients	20 patients

Table 5. Ideal Patient Population Values per Wound Type

In the last chapter, conclusions based on the data presented in this chapter are reviewed and discussed. Future work, including improvements in the study as well as additional conditions to be studied, for this device is advised.

Chapter 5: Conclusions and Future work

Although a portion of this discussion has been presented in the earlier Sections of this thesis, the salient points of the analysis are summarized here to indicate the direct implications of this work for the current clinical practice

As already noted, chronic wounds are a worldwide medical and economic problem, for which there is currently no known cure. Treatment of these wounds is time and labor intensive, placing a large burden on the patient, as well as the medical community. Two of the most commonly treated chronic wounds include venous and diabetic ulcers. Due to the many mechanisms for which these wounds develop, as well as a lack of knowledge as to the real mechanism for why they persist, it is difficult to assess the best treatment and patients must often be followed very closely by a physician. Infection and neglect can at the very least compromise the limb, and in a worst case scenario can cause death due to systemic infection. It was the goal of this limited clinical trial to assess the efficacy of a fully portable and easy to use ultrasound device that would promote healing of these wounds outside clinical environment. The ideal outcome would be to provide chronic wound patients with a device that not only works to heal the wound, but is also safe, portable and pain free so that it can be used at home. This would reduce cost of follow up doctor's visits, as well as promote much needed compliance.

In this study diabetic and venous wounds were treated with low frequency low intensity ultrasound to determine whether or not the wounds treated had a faster rate of healing than those who were not treated. Treatment was administered for 15 minutes once a week for 12 weeks. Patients were recruited from the Drexel University Wound

Healing Center. Once recruited, they were randomly assigned treatment or no treatment, and had an equal chance of being assigned each.

In the venous ulcer group, there were 5 treated and 5 non-treated patients. Two of the treated patients and two of the non-treated patients healed completely, while 3 of the treated and 3 of the non-treated patients did not. The rate of closure was determined via regression modeling, and the 10 patients were added to a previous study of 13 patients. A student's t-test was conducted and determined that there was a significant difference ($p<0.05$) between the treated and non-treated group. This supports the theory that the patients treated with the device had an increased rate of healing and that the device provided treatment for their chronic wound. The power of the hypothesis testing, however, was determined to be approximately ~60%, which is below the desired power (80%) for the test. While this does not negate the hypothesis testing results, it does not provide evidence that adequately complies with FDA guidelines and therefore it is recommended to increase sample size in order to increase the power of the test. Using STATA13™, a desired sample size was assessed as needing to be increased to (n=18), which would bring the statistical power up to desirable 80%.

The diabetic group consisted of 10 patients, randomized into 5 treatment and 5 non-treatment patients. Of the ten patients, one treated patient dropped out due to a heart attack, and another non-treated patient dropped out for unknown reasons. Both of their data was not included for analysis. The remaining 8 patients fully healed by the end of the 12^{th} treatment. Rate of healing was analyzed and was 3 times faster in the treated groups. Using hypothesis testing, this difference was found to be significant ($p<0.01$) between both groups. Power analysis ran through STATA13™ showed a 99% power for

the hypothesis test, which fully supports the hypothesis testing. As a result, the study was able to provide statistically significant evidence that the ultrasound device directly caused diabetic wounds to heal 3 times faster than wounds treated without the ultrasound.

Overall, given the promising results, it is fair to conclude that this wearable device would satisfy the unmet need that is to provide accelerated healing to chronic venous and diabetic ulcer patients. It was also described by the patients as a very easy device to use. Almost all, 90%, of patients reported feeling only as much as a tingle, with no pain associated with the device while it was on at some point during the 12 treatments, while the remaining 10% reported no sensation what-so-ever. It is also important to note, however, that 70% of non-treatment patients also reported feeling a tingle, while the device was not on during the 12 weeks. It is therefore likely that there was a placebo effect of having the device on the wound, or that the sensation of something physically on the wound itself creates a tingle sensation separate from the ultrasound. Either way, it is safe to conclude that this device serves as a pain free option for wound care, with a tingling sensation apparent at the most. Lastly, the device, being fully portable and easy to use, fulfils the requirements discussed in chapter 1 for a patient friendly device. It is reasonable to assume that the combination of affective, portable, user-friendly, and pain free would highly benefit the quality of life, as well as the compliance of this patient population base.

Future work with this device can be expanded to include other types of chronic wounds. In addition to venous and diabetic ulcers, the device could be applied to arterial insufficiency ulcers and pressure ulcers. In addition to ulcers, this device could be assessed for efficacy in healing other conditions, which appear chronic, such as psoriasis,

eczema, and other such skin conditions. It was noted by several patients suffering from edema that the device appeared to "help the swelling". This claim, however, was not investigated, and therefore it would be advantageous to examine it in further studies and determine whether the device can indeed contribute to reducing edema. If confirmed this treatment could then also be applied to a multitude of conditions where limbs have complications due to edema.

Going forward, something that should be controlled for when studying the diabetic population is the levels of hemoglobin A1c (HbA1c) in the blood stream. Hemoglobin is the oxygen-carrying component in blood, and it also is responsible for blood's red color. The component HbA1c is directly related with the amount of glucose in the blood, and is a standard measurement for diabetic patients and can be a great indicator of how controlled their diabetes is. It would be interesting to monitor the HbA1c in the diabetic population and see if there are any correlations between levels of HbA1c and the outcomes of healing and not healing ulcers.

Yet another interesting finding which was observed was the result obtained when the ultrasound was applied on a wound near other non-treated wounds. Two patients were treated with the ultrasound on one of their wounds, while the same leg had other open ulcers located nearby. In both patients, the treated wound healed, while the other surrounding wounds did not. Two other patients were treated for wounds on one leg, while they had another ulcer on the other leg. Again, the treated wounds healed, while the non-treated wounds on the other leg did not. While this phenomenon was not controlled for, or anticipated in the protocol, it would be an ideal measure of the efficacy of the proposed ultrasound therapy. Having patients with multiple wounds would help to

exclude the potential inconsistency associated with tissue variability and individual immune system by – for instance - showing that the wounds treated with the ultrasound device heal faster than those not treated. It could also help to determine the penetration depth of the ultrasound energy. If the ultrasound is found to penetrate through the limb, (quite likely due to the fact that the wavelength is about 75 mm) then it is conceivable that application could be administered on the other side of the limb. This would maximize sterility and further reduce chance of infection.as the wounds would not need to be directly in contact with the device. Thus, it would also reduce the cost by eliminating the need to use Tegaderm dressing and decrease the time needed to prepare the patient treatment.

Another key observance in the wounds, which were treated with the ultrasound was that if the applicator was larger than the wound itself, the wound would exhibit healing by pinching off in the center and becoming two wounds. This phenomenon could be remedied by customizing (tailoring) the applicator's geometry to the size and shape of the individual's wounds. Because the piezoelectric elements are the primary source of the ultrasound, they can be configured in any way, and the embedding resin (see Fig 4) casing could be designed around this. This means that no matter the size or configuration of the wound, a fully customizable applicator could be created.

Lastly, the mechanisms behind the efficacy of the device are still unknown and knowing them – as discussed below- is indispensable in order to customize and personalize the treatment. Whether cell signaling is being conducted via the ultrasound, or bacterial biofilms are being disrupted, it is conceivable that there are many several possible mechanisms by which this ultrasound is aiding in the healing of these wounds.

It would be valuable to know the mechanisms in order to maximize the benefits in the future and customize the device for different ailments. Tissue samples of the wounds prior to and after treatment would be needed to run multiple tests in order to determine mechanism. In vitro studies could also elucidate the effects of ultrasound of different cell types. Currently another Drexel research team is investigating the macrophage interactions and assessing whether the ultrasound is converting M1 macrophages to M2 type macrophages. While M1 macrophages induce the inflammatory response required in acute wounds, they can become counterproductive in chronic wounds. However, the ultrasonically elicited or evoked conversion from M1 to M2, that is into the macrophages, which are associated with tissue repair, may constitute one of the plausible mechanisms by which the ultrasound treatment is working.

As mentioned above, understanding of the mechanisms is paramount for customization of treatment. Different etiologies might call for different treatment recommendations, time intervals, and application frequencies. Knowing the mechanisms of action would allow for customization of ultrasound treatment and could improve outcomes for patients.

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